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Demystifying neuroscience laboratory techniques used to investigate single-gene disorders

ARTICLE

Lindsay A. M. Mizen & **Andrew C. Stanfield**

SUMMARY

There is considerable work being carried out in neuroscientific laboratories to delineate the mechanisms underlying single-gene disorders, particularly those related to intellectual disability and autism spectrum disorder. Many clinicians will have little if any direct experience of this type of work and so find the procedures and terminology difficult to understand. This article describes some of the laboratory techniques used and their increasing relevance to clinical practice. It is pitched for clinicians with little or no laboratory science background.

LEARNING OBJECTIVES

After reading this article you will be able to:

- understand models used in single-gene disorder research
- recognise some of the common methods used in laboratory science to investigate single-gene disorders
- understand how each technique contributes to our understanding of the pathophysiology of intellectual disability and autism spectrum disorder.

KEYWORDS

Single-gene disorders; autism spectrum disorders; intellectual disability; neuroscience; SYNGAP1.

The importance of psychiatrists having an understanding of neuroscience has been increasingly recognised in recent years (Schildkrout 2016; Steele 2019). However, owing to lack of direct personal experience of laboratory work, the language and methods used are daunting for many clinicians. This potentially leaves relevant pathophysiological work impenetrable and clinicians may be less able to read and critically appraise neuroscientific research that could contribute to their understanding of their patients' presentations (Schildkrout 2016). The Royal College of Psychiatrists' Neuroscience Project, funded by the Gatsby Foundation and Wellcome Trust, aims to integrate modern neuroscience into the psychiatry curriculum (https://www.rcpsych.ac.uk/training/neuroscience-in-training/neuroscience). It is hoped that this will address some of these problems.

Within psychiatry, many psychiatric conditions are polygenic, including depression, schizophrenia and bipolar affective disorder. This means that they are mediated by a combination of many different genetic variants, each having a small individual effect. This makes the study of their underlying biology very complex. In the psychiatry of intellectual disability, single-gene disorders are much more common, therefore offering an advantage when it comes to understanding the pathophysiology of the conditions presenting in clinic. The Simons Foundation Autism Research Initiative (2019) now associates over 900 genes with autism spectrum disorder and by 2015 over 700 genes related to intellectual disability had been identified, with that figure predicted to rise (Vissers 2015). The Deciphering Developmental Disorders multicentre study recruited over 12 000 children who had marked developmental delay, but no identified cause. It was estimated that 42% carried a genetic variant that was felt to underlie their condition (McRae 2017). It is important to note that the

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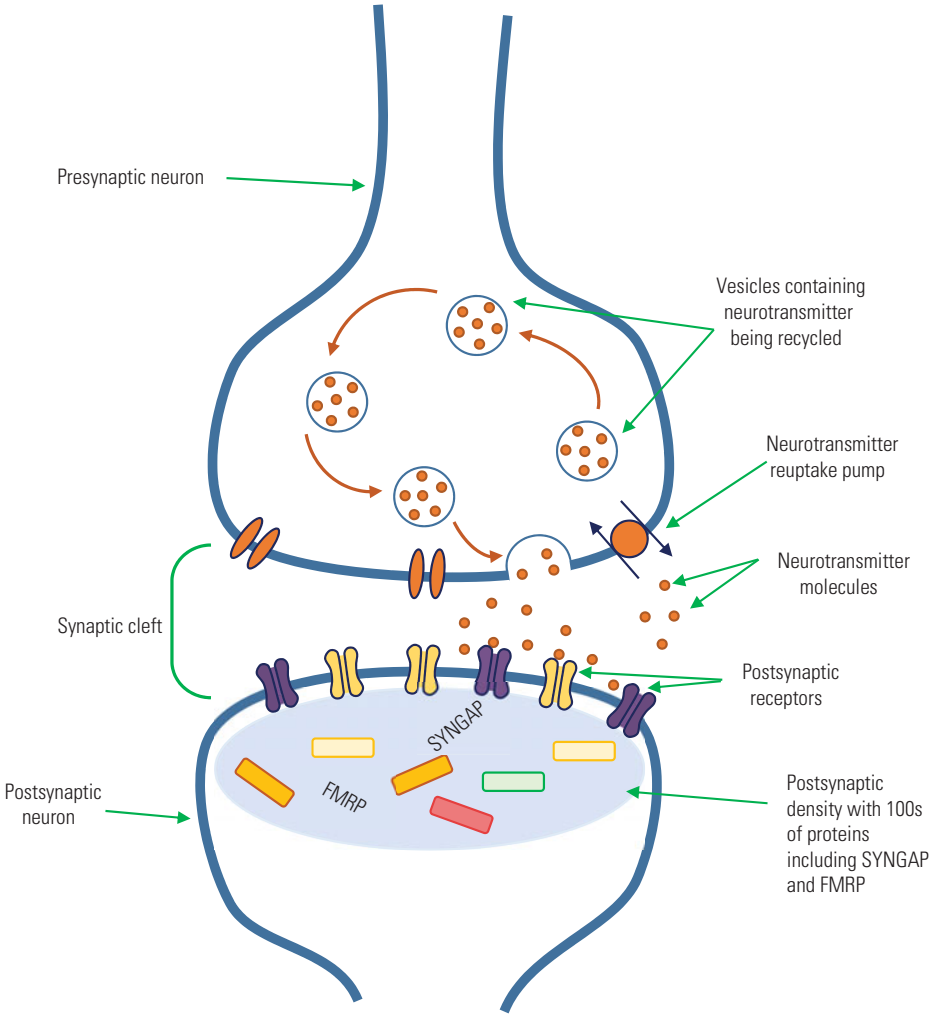

FIG 1 Schematic diagram of a synapse.

sequencing techniques used to find these variants have only recently become widely available. Consequently, many people with intellectual disability remain undiagnosed and the clinical impact of these findings is just beginning to be felt.

It is now relatively common for clinicians to receive a report of a genetic diagnosis about which they have little or no knowledge. Often, much of the research about it has used basic neuroscience techniques in animal studies and research about the presentation in humans may be limited. Therefore, being able to understand and discuss the laboratory science behind these conditions is empowering, and work on the signs and symptoms of these disorders using animal models can be relevant to the presentation in patients. Furthermore, as neuroscientific research starts to delineate the pathophysiology, potential pharmacological targets are being identified, which of course opens the door to new drug treatments. Hence, there is a need for clinicians to understand the basic science research as much as possible.

Using language suitable for a clinical audience, this article describes some of the techniques that are available to neuroscientists investigating single-gene disorders. The particular example of *SYNGAP1*-related intellectual disability will be used to illustrate the relevance of techniques to single-gene disorders presenting in clinic.

SYNGAP1-related intellectual disability

SYNGAP1 is a gene that codes for a protein called SYNGAP1, which is abundant at synapses, the clefts between neurons where electrical activity is transferred from one neuron to another (Fig. 1).

The electrical activity reaching the synapse induces the release of neurotransmitter molecules from vesicles, which then diffuse across the cleft and bind to receptor molecules in the postsynaptic membrane. The more receptors present, the stronger the synaptic signal. These receptors are associated with the hundreds of proteins in the so-called 'post-synaptic density' (PSD). The Syngap1 protein was

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identified in 1998 as one of the proteins in the PSD (Chen 1998; Kim 1998). It has been found by many researchers to regulate various postsynaptic biochemical pathways by acting like a brake (Jeyabalan 2016). *Syngap1* gene mutations in rodents[a] have been associated with hyperactivity, seizures and deficits in learning, memory and social functioning (Jeyabalan 2016).

The first link between people with intellectual disability and *SYNGAP1* mutations was made long after the protein was being studied in laboratories (Hamdan 2009). Since then many studies have found that variants (mutations) in the *SYNGAP1* gene in people are associated with intellectual disability. The level of cognitive impairment is typically moderate to severe and almost all of those affected have epilepsy (Mignot 2016); this mirrors animal study findings. The epilepsy can be severe and notably can present with eating-induced seizures (von Stülpnagel 2019). Around 50% of individuals affected also meet the criteria for a diagnosis of autism spectrum disorder, and the other symptoms associated with *SYNGAP1* pathogenic variants include hypotonia, gait abnormalities, high pain threshold and sleep disturbance (Parker 2015; Mignot 2016; Vlaskamp 2019).

Affected individuals and families are keen to understand as much as they can about how *SYNGAP1* variants cause symptoms and to seek a cure. Bridge the Gap, the Syngap Education and Research Foundation, is a non-profit organisation whose mission is 'to serve, educate and fund research for families coping with the effects of SYNGAP mutations' (https://bridgesyngap.org/). As is the case with many support groups and foundations for people affected by genetic conditions, those involved with Bridge the Gap work closely with laboratory and clinical researchers to better understand *SYNGAP1*-related intellectual disability and to participate in research. As a result, they often have a comprehensive understanding of the basic science research relating to the genetic condition of interest to them. Clearly, it is therefore advantageous for psychiatrists working with individuals with genetic diagnoses to understand them too. Although it is not possible for psychiatrists to familiarise themselves with all the genetic diagnoses that will present in clinic, they need to be able to seek and comprehend the available research relevant to a given patient. This will involve an appreciation of the basic scientific approaches used to investigate these conditions.

Types of model used to study single-gene disorders in the laboratory

Different types of scientific 'model' can be used to study single-gene disorders by trying to recapitulate the human disorder through studying the function of the gene. Two common models are rodent models (primarily mice or rats) and induced pluripotent stem cells (iPSCs).

Rodent models

Rodent models are particularly useful for a gene like *SYNGAP1* as their genetic code is 'highly conserved' across species, meaning that the *SYNGAP1* DNA sequence is very similar in mice, rats and humans. One of the limitations of pathophysiological work in humans with mental disorders is the relative lack of access to the organ of interest (the brain). However, this is not the case in rodents. Their DNA can be manipulated to change the function of genes associated with intellectual disability and autism spectrum disorder in humans, allowing the effects in the brain to be studied.

This manipulation can be done in several ways, usually with the aim of activating, inactivating or modifying a gene's function. In addition to altering a gene so that the protein product is manipulated throughout an organism, it is also possible to alter the gene in such a way that it only has an effect in a certain population of cells; this allows the study of protein function in these particular cells. Hence, it may be possible to study the role of a protein in the brain without manipulating its role in other parts of the body.

There are myriad methods to manipulate DNA in rodents and it is beyond the scope of this article to detail them. Often, several different rodent models of the same condition will exist because slightly different methods were used to alter their DNA. For example, three different mouse models of *SYNGAP1*-related intellectual disability were engineered by disrupting different parts of the *Syngap1* gene. One of these was constructed so that the alteration in the gene could be 'switched' on or off in the presence of an enzyme called Cre recombinase to compare the biology in different states in the same animal. The other mice were designed to be 'knockouts', meaning that the genetic alteration aimed to be so severe that the protein product (Syngap1) was not made at all.

It is also possible to manipulate genomes so that a protein is still produced but is in some way altered. This is true of the first rat model of *SYNGAP1*-related intellectual disability, which had a large deletion in the middle of the gene so that a section of the protein was missing.

A highly specific method of genome manipulation that is increasingly used is known as CRISPR-Cas9, an accessible explanation of which has been published online by the US National Library of Medicine (2020). Essentially, this is a way of targeting a very specific section of DNA and is faster, cheaper, more accurate and more efficient than earlier genome editing

a. We follow the convention of showing human and rodent genes differently. Thus, the human gene is shown as *SYNGAP1* and protein as SYNGAP1, the rodent gene as *Syngap1* and protein as Syngap1.

methods. It is based on a system used by bacteria and the idea is that a small piece of RNA that binds to the sequence of DNA of interest is generated and associated with an enzyme (Cas9), which cuts the DNA at that point. This allows the addition, deletion or precise change of portions of DNA.

Induced pluripotent stem cells (iPSCs)

Pluripotent stem cells are stem cells that can differentiate into all the various cell types in the body. In the mid-2000s, researchers in Japan developed a way to return fibroblast skin cells that have already differentiated back to their pluripotent state (Takahashi 2006). They called these cells 'induced pluripotent stem cells' (iPSCs). The iPSCs can be grown on and encouraged to differentiate into new cell types. It is now possible to take somatic cells (e.g. from skin, blood or even urine samples), reprogram them into stem cells and then, in the right conditions, grow them on into neurons. This has particular utility in neuropsychiatric conditions, where it is not typically possible to biopsy the brain to study it (Dolmetsch 2013). Hence, skin biopsies can be taken from people with a particular condition to go on to investigate the function of their neurons and to test potential drugs (Hettige 2018).

Often, iPSC lines are produced from individuals with an identified genetic variant that is causative of, or puts them at risk of, a specific condition. Some causes of intellectual disability are being investigated using iPSCs, such as fragile-X syndrome and Rett syndrome (Brito 2018). To our knowledge, no studies have been published to date of such work in *SYNGAP1*-related intellectual disability.

Introduction to laboratory-based research techniques

Psychiatric conditions can be conceptualised on various levels, from genetics and cell biology to complex neuronal circuitry. Each level leads to the recognisable anatomy of the brain and its function, hence influencing behaviour and mental health and illness (van den Heuvel 2019). This can be investigated in the laboratory in many ways, some of which are described below.

Studying biochemical pathways

The benefits of understanding the mechanistic pathways related to human disease are widely recognised, with prominent examples such as the metabolism of alcohol in the liver, the insulin regulation pathways relevant to diabetes and the role of specific hormones in certain forms of cancer (Barr 2018).

In autism spectrum disorder and intellectual disability it has been established that various single-gene disorders are linked to the function of synapses (Krumm 2014) and hence there may be convergence of the mechanisms by which they influence the human condition. An increasing understanding of this convergence allows translational research to focus on the areas felt to be most fruitful for the development of therapeutic strategies. It is hoped that treatments might be engineered that can be used for multiple genetic diagnoses.

For example, the pathophysiology of *SYNGAP1*-related intellectual disability has similarities with that of fragile-X syndrome. Fragile-X syndrome results from an absence of the fragile-X mental retardation protein (FMRP) due to mutations in the *FMR1* gene. Many of the biochemical pathways which FMRP normally acts on have been studied and there is overlap with Syngap1 protein function. FMRP has various functions, including a brake-like regulation of certain excitatory glutamate receptors (metabotropic glutamate receptor type 5 (mGluR5) to be precise). Therefore, in rodents lacking FMRP, there is overactivity of such receptors, which has various effects (Huber 2002). For example, in the hippocampus this leads to a downregulation of signalling (long-term depression) (Qin 2005). Long-term depression, along with its opposing counterpart long-term potentiation, is accepted as a cellular correlate of learning and memory. Experiments in mice with *Syngap1* mutations have now also shown increased hippocampal protein synthesis and exaggerated long-term depression, also seen in mouse models of fragile-X syndrome (Barnes 2015).

This is important work as the activity of mGluR5 receptors and/or the cascade of biochemical pathways that they are involved in can be targeted with drugs, including some already in human use for other purposes. For example, as you will be aware, the primary use for statins currently is to lower cholesterol via their effect on an enzyme called hydroxymethyl glutaryl coenzyme A (HMG-CoA) reductase. However, the biochemical pathways they affect also link to those downstream of mGluR5. Therefore, research has been carried out with one such drug in particular – lovastatin – which has been shown to normalise the excess cellular protein synthesis and exaggerated long-term depression in mouse models of fragile-X syndrome and *SYNGAP1*-related intellectual disability (Barnes 2015). A small open-label study of lovastatin's use in people with fragile-X syndrome was tentatively positive, although there is a need for large-scale randomised trials (Çaku 2014). This need for robust evidence of efficacy is particularly true because other classes of drug, such as mGluR5 antagonists (e.g. mavoglurant) and also gamma-aminobutyric acid (GABA) agonists (e.g. arbaclofen), predicted to ameliorate symptoms of fragile-X syndrome owing to their known biochemical effects, have to date not shown any clear effect

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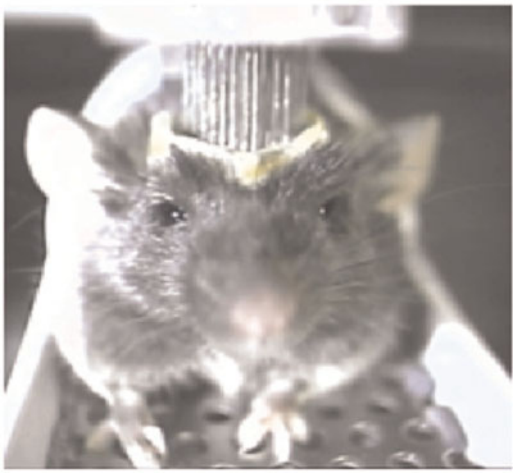

FIG 2 Mouse with surface electroencephalogram (EEG) electrodes attached. (Adapted from Sheybani et al, 2018. Reproduced with permission from *The Journal of Neuroscience*. The full figure is available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6705908/figure/F1.)

in people. Researchers are continuing to try to understand the reasons for this. Theories include a true lack of efficacy, the development of drug tolerance or problems with the research design such as the age of the participants, length of the trials or type of outcome measures used (Erickson 2017).

Investigating the electrical activity of the brain

Electroencephalography (EEG) is of course widely used as an investigation in people with neurological conditions such as epilepsy. People with *SYNGAP1* variants have been shown to have a variety of epileptiform activity on EEG as well as other abnormalities (Gamache 2020). EEG can also be done in rodents and, as with humans, pairing it with a video recording of the subject is particularly informative, especially if seizure activity is suspected. Surface electrodes can be attached to the skull of a rodent under anaesthesia (Fig. 2) or electrodes can be implanted into the brain. Recording equipment can then be attached and EEG traces recorded while they are asleep or awake and behaving. 'Behaving' sometimes refers to times when no specific demands are placed on the animals and sometimes refers to them undertaking an active behavioural task. Such experiments have shown that, similar to affected humans, epileptiform discharges are present in mice with *Syngap1* mutations (Clement 2012; Ozkan 2014).

Neuronal electrical activity can also be explored in ways that cannot be replicated in humans because brain tissue is required. In rodents this is commonly done by recording from brain cells grown in a dish (cultured cells) or from brain slices (Fig. 3).

Some genetic conditions, including *Syngap1* mutations, are lethal in rodents early in life if both copies of the gene are mutated. This clearly limits the opportunity to conduct research into them. However, cultured neurons from these animals often survive when collected in the perinatal period and electrical recordings can still be made from them (they have other uses too). The lethality of *Syngap1* mutations in rodents is interesting as to date all people identified with *SYNGAP1*-related intellectual disability have only one mutated copy of the gene, possibly suggesting that homozygosity (where both copies are mutated) is incompatible with life in humans too.

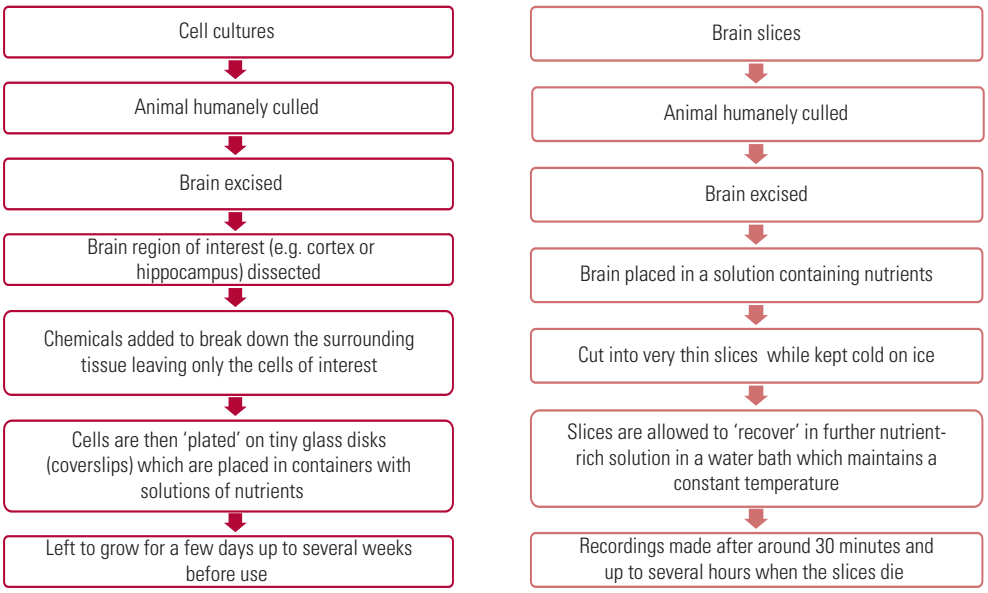

FIG 3 Preparation of rodent cell cultures and brain slices.

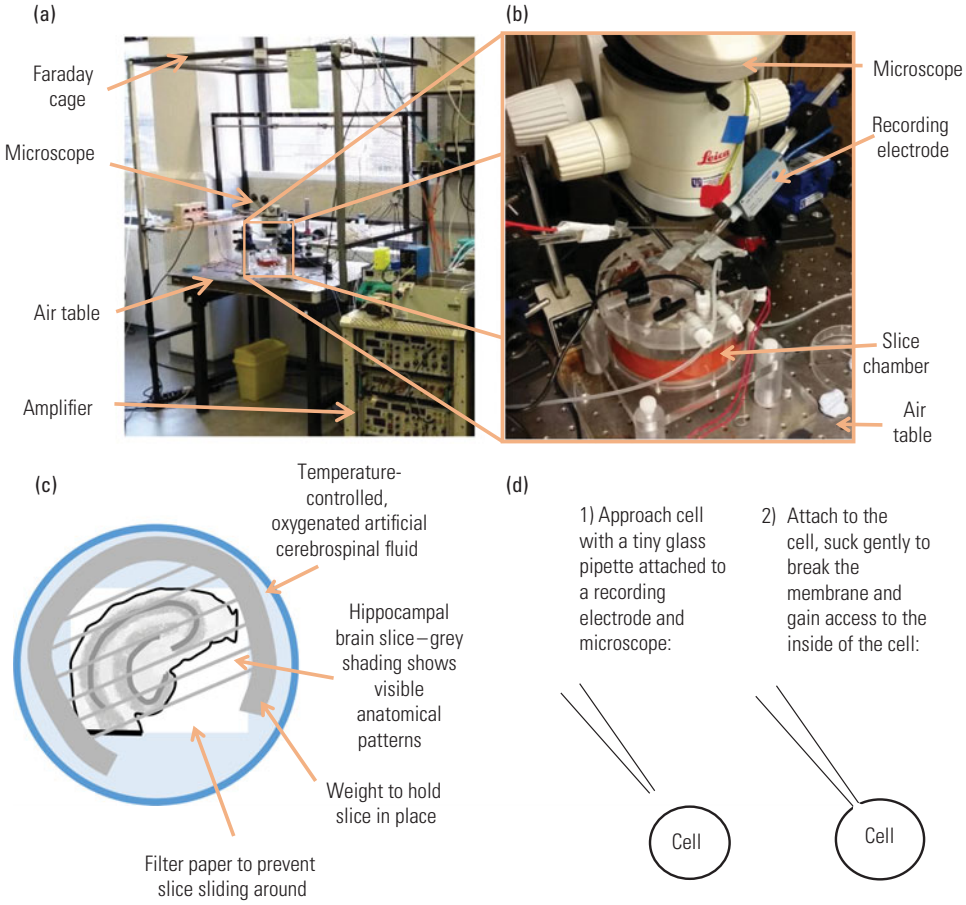

FIG 4 Electrophysiology equipment. (a) Microscope and slice chamber with air table and Faraday cage to reduce electrical interference. (b) Microscope, slice chamber and recording electrode, which connects to an amplifier and computer. (c) Schematic of view into slice chamber. (d) Schematic of gaining access to a single cell to make a recording.

For electrophysiological recordings from single cells or local cell networks, the electrical currents studied are tiny. For comparison, a typical kitchen kettle draws around 13 amps of current, whereas single-cell or local cell network recordings are in the region of a few picoamps (13 amps is equivalent to 13 000 000 000 000 picoamps, illustrating just how small these currents are). Hence, the recordings are very easily distorted by movement or electrical interference. To minimise these, the recordings must be made on equipment sitting on an air table (floating on a cushion of air) within a Faraday cage which blocks electromagnetic fields. A microscope sits on the air table with either space for a cell culture coverslip below or a temperature-controlled chamber for a brain slice. An example of such an electrophysiology 'rig' is shown in Fig. 4(a) and (b). Tiny glass pipettes are attached to electrodes and used to pierce the tissue to make recordings generally in the region of interest or to target individual cells (Fig. 4(c)). When recording from single cells, the end of the pipette effectively becomes part of the cell membrane to prevent the cell from bursting (Fig. 4(d)). A second electrode can be used to electrically stimulate areas of the tissue to see how this affects the function of the regions/cells recorded from. 'Passive' recordings are also done, which record properties such as the resistance of the cell membrane without external stimulation.

Thus, many different electrical properties of the brain can be recorded and compared between mutant and wild-type animals. At a single-cell level the most instantly recognisable for clinicians is probably the action potential, the mode by which electrical activity is propagated through neurons. The number of action potentials in a given period gives an idea of how active (or excitable) the cell is, and different aspects of action potentials (how fast they rise and fall, how long they last, etc.) can be analysed too. It is also possible to study the strength of signal passed from one neuron to another by stimulating the cells or networks in a certain way; this is how the long-term depression described above is studied.

Because of the Syngap1 protein's brake-like effect on biochemical pathways, in its absence

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changes in synaptic transmission have been identified, including:

- a reduction in long-term potentiation, i.e. a reduction in the normally raised transmission following electrical stimulation, which is a correlate of learning (Ozkan 2014)
- exaggerated long-term depression (as mentioned above) (Barnes 2015)
- increased size and frequency of certain single-cell electrical currents suggestive of greater excitability (Vazquez 2004; Rumbaugh 2006; Ozkan 2014).

This type of work is of interest given the cognitive deficits seen in patients and theories of alterations in excitation or inhibition as a cause of conditions such as autism spectrum disorder. However, as with many genetic conditions, results from *Syngap1* rodent work vary between different brain areas, cell types and animals of different ages, so much more work is needed to fully understand its role.

Studying behaviour

Various paradigms exist to study aspects of an animal's behaviour that may correlate with the presentation of intellectual disability and autism spectrum disorder in humans. For example, tests of their learning and memory, anxiety and social interest.

A very well-accepted way of testing learning and memory in rodents is using the Morris water maze (Morris 1981). The idea is that the rodents are placed in a pool of opaque water in which there is a platform above the water level that they can locate to escape the water (Fig. 5). After they have been trained to know where the platform is, it is changed to a submerged platform that they have to search for. Parameters around how long it takes them to locate the platform and how well they search for it can be measured. The rodents are placed in the pool from the same place each time and this entry point can be changed after a while to investigate how well they learn to reorient themselves. The position of the platform can also be changed to see how well they move on from searching in the original area to other quadrants of the pool.

Various other mazes are also routinely used, such as the elevated plus maze, which consists of two narrow walkways arranged in a cross shape and supported about 50 cm above the ground. Two of its 'arms' are open and two have side walls (Fig. 5). As rodents have a natural aversion to open spaces, the number of times a rodent enters an open arm and/or the total time spent in open arms is used as a marker of anxiety. Another common way to test anxiety is to place a rodent in an empty chamber and measure how much time it spends out in the open compared with staying close to the walls. This is known as the open field test. Comparing animals with and without single gene mutations in these mazes can therefore be informative. Such tests also enable the effects of pharmacological agents on the animals' presentation to be studied.

Rodents have a strong tendency to explore novel objects, and when they are presented with something they have not seen before, they will spend more time exploring this than familiar objects. The ability of mutant and wild-type rodents to distinguish between familiar and novel objects can therefore be examined. These tests can be made more challenging by investigating whether the animals can recognise the context (place) in which they saw an object, for example what type of cage they were in. They should consider an object as novel only if it is in a new position in a new cage (Fig. 6). As the complexity of such tests increases, rats need to be older before they can successfully complete the task.

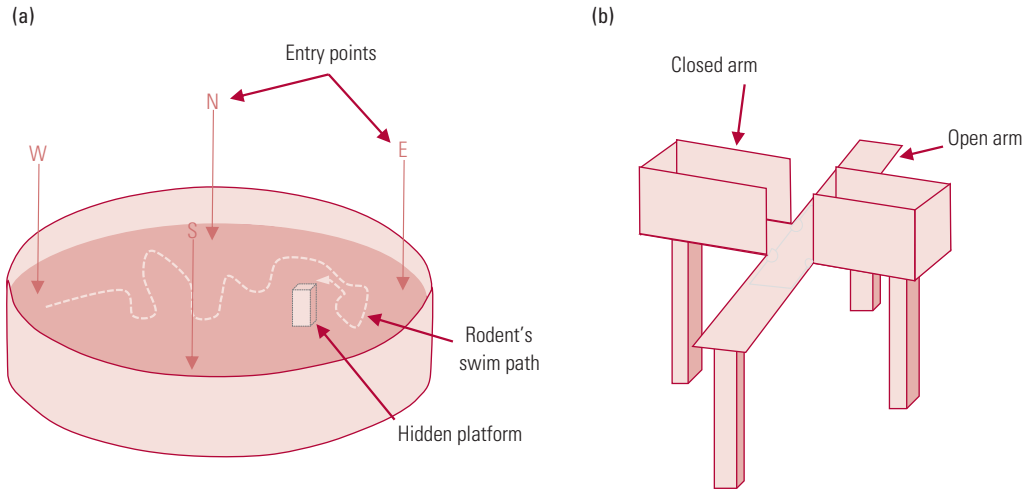

FIG 5 Schematic of two mazes commonly used in rodent work. (a) Morris water maze. (b) Elevated plus maze.

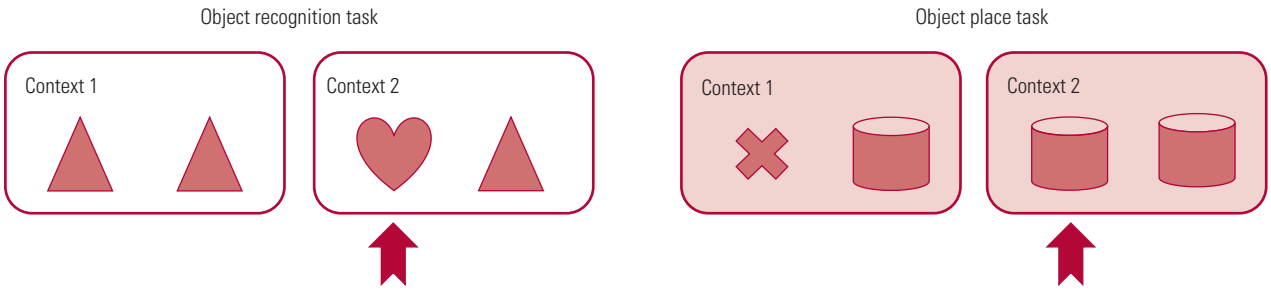

FIG 6 Diagram of the object recognition and object place tasks. In each experiment, the rodent is first placed in context 1 and later moved to context 2. In the object recognition experiment, the rodent should detect the heart in context 2 as novel. In the more difficult object place experiment, it should detect the left-hand cylinder as novel in context 2, as it was not in this location before.

Using these or other behavioural tests, rodents with *Syngap1* mutations have been shown to have learning deficits and decreased anxiety (Jeyabalan 2016). Results are awaited of such object–place–context experiments (as they are known) in rats with *Syngap1* mutations at the Patrick Wild Centre at the University of Edinburgh. However, similar experiments by the same research team have shown that animals with mutations that model fragile-X syndrome took longer to learn the tasks and, in the most complicated paradigms, did not learn them at all despite reaching adulthood (Till 2015). Furthermore, the researchers found that lovastatin improved the learning of these rats even when given for only a brief period in early life, showing the utility of behavioural paradigms in the testing of potential treatments.

Given that social behaviour can be affected by intellectual disability and autism spectrum disorder, this is something else that is commonly studied in rodents with single-gene disorders related to these conditions. Rodents will usually spend more time interacting with a fellow rodent than an inanimate object. The three-chamber sociability test allows researchers to investigate this. The test rodent is placed in a central chamber but can move freely to the edge of adjacent left and right chambers. In one there is another rodent and in the other an inanimate object and the proportion of time spent in the vicinity of each can be measured. The chambers can also be used to test whether rodents are able to remember other rodents they have met before, because they should spend more time interacting with a 'novel' animal. Deficits in these social interactions have been found in rodents with *Syngap1* mutations (Jeyabalan 2016).

Once again, although rodent behaviour is not a direct proxy for the human condition, these tests are well-validated measures and do give some information regarding cognition, memory and sociability, which are affected in humans with single-gene disorders.

Brain imaging

Similar to electrophysiology, imaging can be considered at many levels. As with humans, it is possible to conduct functional magnetic resonance imaging (fMRI) of rodent brains, allowing direct comparisons to be made in certain brain areas. This form of imaging measures changes in blood flow in the brain, an increase in which is indicative of greater activity in that area. These scans can be done at rest or when carrying out a specific task. In rats, a fear conditioning paradigm has been developed at the University of Edinburgh that takes advantage of Pavlov's classical conditioning (Brydges 2013). As a brief recap, this is the form of learning in which an individual learns to associate a neutral stimulus with a particular outcome and this leads to a behavioural response. In the fear conditioning paradigm, the unconditioned stimulus is a mild foot shock, to which the rodent's natural response is to freeze (unconditioned response). The rodents are taught the association between the foot shock and a flashing light (conditioned stimulus) in advance of brain scanning. They therefore learn to freeze in response to the presentation of the light alone (conditioned response). The task is repeated several times to reinforce the association of the flashing light with the foot shock. The animals are then anaesthetised to be placed in an fMRI machine designed for rodents to which they have been previously habituated. Once they wake up, they are again presented with a flashing light and the changes in the brain activity in response to fear can be imaged (Fig. 7).

Moving away from fMRI, at the single-neuron level it is possible to image the morphology of neurons, particularly their dendritic spines. These are small protrusions along the neuron (Fig. 8(a) and (b)) which are important as they are the sites where synapses form with other neurons and because synaptic efficacy has an impact on learning and memory and other cognitive processes. Analysis of *Syngap1* neurons in this way has shown some

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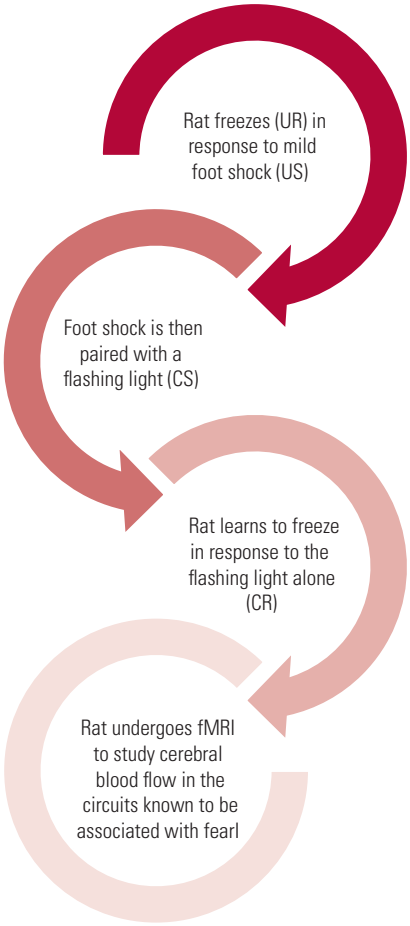

FIG 7 A classical conditioning paradigm used in rat functional magnetic resonance imaging (fMRI). CR, conditioned response; CS, conditioned stimulus; UR, unconditioned response; US, unconditioned stimulus.

BOX 1 Key messages for clinicians

- Single-gene disorders causing intellectual disability and autism spectrum disorder are increasingly being diagnosed
- Consequently, more patients, families and carers are seeking information about them and psychiatrists need to be acquainted with the research, much of which is laboratory based
- Research identifying the function of the protein coded for by a specific gene allows greater understanding of the pathophysiology in the brain
- This pathophysiology can be studied in the laboratory using a multitude of techniques and at different levels of brain function
- Such studies can lead to the identification of psychopharmacological targets or behavioural markers, leading to new treatments and ways to evaluate their efficacy

changes in the head and neck size of dendritic spines in *Syngap*$^{+/-}$ mice (knockout mice) (Carlisle 2008; Clement 2012; Barnes 2015). Furthermore, in fragile-X syndrome rodent models, the loss of the FMRP protein has been shown to result in changes in the stability of spines and proper spine pruning during development (Gipson 2017).

Two common ways to image single cells are as follows:

- the use of similar techniques and equipment to electrophysiology to gain access to the inside of the neuron so that dye can be injected in and a microscope used to examine the cell structure
- the use of fluorescent chemicals to 'label' the cell so that they can be analysed with extremely powerful microscopes (Fig. 8(c)).

Calcium imaging is another technique with which to image neurons. A fluorescent dye specifically designed to bind to intracellular calcium is modified to make it lipophilic so that it can cross the cell

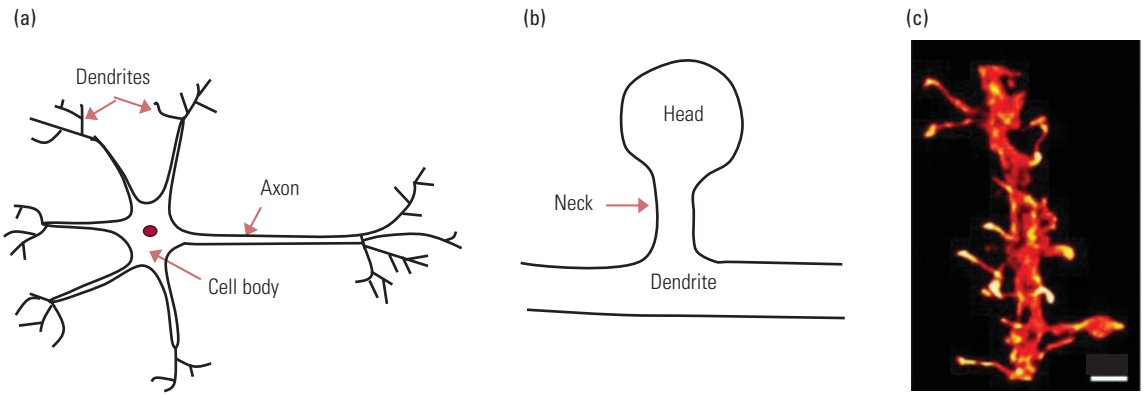

FIG 8 Imaging of a single neuron. (a) Anatomy of a neuron. (b) Schematic of a dendritic spine. (c) High-power microscopy image of a neuron with dendritic spines clearly visible. (Part (c) is adapted from Wijetunge et al, 2014. Reproduced with permission from *The Journal of Neuroscience*. The full figure is available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4004821/figure/F1/.)

MCQ answers
1 c 2 b 3 a 4 b 5 e

membrane. Once inside, it glows when calcium binds to it and this fluorescence can be detected and quantified using special microscopes. With certain forms of microscope it is even possible to see where within the cell the calcium signal is coming from down to the level of the synaptic boutons (the presynaptic part of a synapse). This is important research as calcium is involved in the release of neurotransmitters from presynaptic vesicles and also in alterations in gene expression within cells. Measuring calcium signals is therefore seen as a functional read-out of the electrical activity of neurons.

Finally, it is also possible to take time-lapse images of cells, which when used with fluorescently tagged proteins enable researchers to 'watch' the movement of these proteins within a single cell. This technique has shown that stimulating the cell in a certain way results in the Syngap1 protein dispersing away from dendritic spines (and therefore synapses) (Araki 2015).

Conclusions

Laboratory neuroscientific research is contributing significantly to our understanding of single-gene disorders underlying intellectual disability and autism spectrum disorder. Combining different techniques helps scientists to study the pathophysiology from subcellular through to whole-organism level. Considering different strands of research in isolation gives a far less rich picture and their relevance to clinical practice could be questioned. Indeed, knowledge at the cellular or local-network level could, on face value, be somewhat dismissed by clinicians, but it is often this level of understanding that leads to theories regarding pharmacology, brain network function or changes in behaviour that can then be tested. Often the process works the other way too, for example delineating the mechanism of medications that have incidentally been found to be therapeutic in patients.

Therefore, identifying specific phenotypes through basic neuroscience allows some comparison to be made with the human condition, albeit with the caveat of the significant species difference between animals and humans. Perhaps more importantly, identifying specific signatures or patterns related to a condition in animals (e.g. changes in neuronal electrical activity, abnormal behaviour or changes in fMRI signal) provide translational markers against which to test new therapeutic strategies. As questions are increasingly asked about the clinical presentation of genetic conditions and as laboratory research works towards new treatments, it is important for clinicians to understand the relevant basic neuroscience. Box 1 summarises key messages from this research.

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Author contributions

Both authors conceived the idea and planned the structure of this article. L.A.M.M. prepared the manuscript, which was reviewed by A.C.S.

Declaration of interest

None.

ICMJE forms are in the supplementary material, available online at https://doi.org/10.1192/bja.2020.30.

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MCQs

Select the single best option for each question stem

1 The percentage of children with significant developmental delay of unknown cause in whom a genetic cause may now be identified is:
- a up to 20%
- b 30–40%
- c 40–50%
- d 50–60%
- e over 60%.

2 The biochemical pathways that the SYNGAP1 protein is known to regulate overlap with those of:
- a Down syndrome
- b fragile-X syndrome
- c Williams syndrome
- d velocardiofacial syndrome
- e Smith–Magenis syndrome.

3 In the imaging of rat brain fear circuits, researchers can use the learning theory of:
- a Pavlov
- b Skinner
- c Bandura
- d Watson
- e Thorndike.

4 Functional magnetic resonance imaging (fMRI) of the brain can be used to measure:
- a calcium changes
- b changes in blood flow
- c electrical activity
- d water distribution
- e neuronal morphology.

5 An accepted cellular correlate of learning and memory is:
- a neurotransmitter release
- b action potential
- c long-term prolongation
- d calcium-binding
- e long-term depression.